The EMBO Journal vol.9 no.9 pp.2891 - 2898, 1990

Yeast MIG1 repressor is related to the mammalian early growth response and Wilms' tumour finger proteins

Jan Olof Nehlin and Hans Ronne

Ludwig Institute for Cancer Research, Uppsala Branch, Biomedical Center, Box 595, S-751 23 Uppsala, Sweden

Communicated by C.-H.Heldin

We have cloned a yeast gene, *MIG1*, which encodes a C_2H_2 zinc finger protein involved in glucose repression. The fingers of MIG1 are very similar to those present in the mammalian Egr finger proteins, which are induced during the early growth response, and also to the finger protein encoded by a human gene that is deleted in Wilms' tumour cells. MIG1 protein binds to two sites in the upstream region of *SUC2*, a yeast gene that is repressed by glucose. The MIG1 sites closely resemble the sequence recognized by the Egr proteins. Thus, finger proteins that are similar in both amino acid sequence and DNA specificity are involved in the response of yeast to glucose, and in the mammalian early growth response.

Key words: early growth response/glucose repression/Wilms' tumour/yeast/zinc finger protein

Introduction

While positive control of eukaryotic genes has been studied in some detail, much less is known about negative control of transcription (Levine and Manley, 1989). In the yeast *Saccharomyces cerevisiae*, a ubiquitous form of negative control is glucose repression. Thus, a large number of yeast genes involved in carbohydrate catabolism, gluconeogenesis and respiration are repressed by glucose (Entian, 1986; Gancedo and Gancedo, 1986; Carlson, 1987). This is part of a complex response in which the cell adapts for rapid growth on glucose by shutting down certain metabolic pathways and activating others. A number of mutations that affect glucose repression of different yeast genes have been isolated, but the mechanism is still poorly understood. One set of genes for which glucose repression has been studied is the *GAL* genes (Johnston, 1987). These genes are subject to both galactose induction and glucose repression. A simpler model for studying glucose repression is the *SUC2* gene, which encodes the sucrose- and raffinose-degrading enzyme invertase. *SUC2* expression is controlled only by glucose, and several genes involved in regulating *SUC2* have been identified (Entian, 1986; Carlson, 1987).

We have used a new approach to investigate negative control of transcription. The yeast *GAL1* promoter was used for lethal overexpression of yeast cAMP dependent protein kinase. A multicopy yeast library was then screened for plasmids that could rescue these cells by turning off the *GAL1* promoter (Nehlin *et al.*, 1989). Several new genes were cloned, one of which, *MIG1*, is described below. *MIG1* encodes a C_2H_2 zinc finger protein with fingers that are very similar to those present in the mammalian early growth response and Wilms' tumour finger proteins. We have found that MIG1 is a DNA binding protein involved in glucose repression, and binds to two sites in the *SUC2* upstream region.

Results

Cloning and mapping of the MIG1 gene

The *MIG1* gene was isolated as a multicopy inhibitor of the *GAL1* promoter. To clone such inhibitors, we used a plasmid in which the *TPK2* gene, encoding yeast cAMP dependent protein kinase (Toda *et al.*, 1987) is transcribed from the *GAL1* promoter (Johnston and Davis, 1984). Induction of the promoter by galactose kills cells harbouring this plasmid, since kinase overexpression is lethal. A yeast strain with a chromosomally integrated copy of the plasmid was transformed with a yeast genomic library made in the high copy number vector pHR81 (Nehlin *et al.*, 1989). To find plasmids that inhibit *GAL* gene expression, we screened cells for the ability to grow on galactose. Such selection was possible since aerobic growth on galactose does not require the *GAL* gene products. Colonies in which the *GAL* genes were turned off were then identified by their inability to grow on galactose in the presence of ethidium bromide, a drug which inhibits aerobic growth (Johnston and Davis, 1984). A large number of plasmids that interfered with the *GAL1* promoter were cloned in this way. Many of these plasmids contained promoters of other *GAL* genes, which inhibit *GAL1* expression by promoter competition (Nehlin *et al.*, 1989). However, 19 plasmids contained a new gene which we call *MIG1*, for Multicopy Inhibitor of *GAL* gene expression. One plasmid, pMIG1, was chosen for further studies (Figure 1).

The *MIG1* gene was located to chromosome 7 in a Southern blot of yeast DNA separated on a CHEF gel (Chu *et al.*, 1986). To map the gene, we followed *LEU2* or *URA3* disruptions of *MIG1* in multipoint crosses to *ade5*, *met13*, *cyh2*, *rad6* and *trp5*. We found that *MIG1* maps between *trp5* and *rad6*, 5.4 cM from the former marker (Table II). Map distances were in good agreement with the genetic map (Mortimer *et al.*, 1989) except for the fact that *rad6* mapped to a point midway between *trp5* and *cyh2*, rather than close to *trp5*.

MIG1 encodes a C_2H_2 zinc finger protein

The *MIG1* gene was located within pMIG1 by deletion mapping, and the sequence of the gene was determined (Figure 2). *MIG1* has an open reading frame of 504 codons, encoding a 56 kd protein. The *MIG1* upstream region does not contain a classical TATA box, but a TATTTA motif is found at position −163. This sequence was recently shown to be an efficient TATA box in yeast (Harbury and Struhl, 1989). A computer search of the NBRF database revealed that the MIG1 protein is related to transcription factor IIIA (Ginsberg *et al.*, 1984). The similarity is located in the amino

terminus of *MIG1*, which has two C_2H_2 zinc finger motifs similar to those found in TFIIIA and several other eukaryotic transcription factors (Klug and Rhodes, 1987; Evans and Hollenberg, 1988). This suggested that MIG1 could be a DNA binding regulatory protein.

The fingers of MIG1 are very similar to those encoded by two mammalian genes (Figure 3). One gene is known as *Krox-20* (Chavrier *et al.*, 1988) or *Egr-2* (Joseph *et al.*, 1988); the other as *Krox-24* (Lemaire *et al.*, 1988), *Egr-1* (Sukhatme *et al.*, 1988), *zif268* (Christy *et al.*, 1988) or *NGFI-A* (Milbrandt, 1987). Both genes, subsequently referred as the *Egr* genes, belong to the immediate early growth response genes, which are induced within a few minutes following mitogenic stimulation. It has therefore been suggested that Egr proteins could be involved in the control of cell proliferation. Krox-20 is also expressed in a segment-specific way in the developing central nervous system (Wilkinson *et al.*, 1989). A third recently described finger protein, which is similar to both MIG1 and the Egr proteins, is encoded by a human gene that is deleted in Wilms' tumour cells (Call *et al.*, 1990; Gessler *et al.*, 1990). Finally, it has also been noted (Chavrier *et al.*, 1988) that the fingers of the Egr proteins are quite similar to those of transcription factor Sp1 (Kadonaga *et al.*, 1987).

While the fingers of all these proteins are highly conserved, little similarity is seen elsewhere. A comparison must therefore rely on an alignment of the finger motifs (Figure 3). This is complicated by the fact that the number of fingers varies between proteins. Moreover, the fingers within each protein differ with respect to sequence conservation. In particular, the amino terminal finger is more divergent in several of the proteins, including MIG1. We therefore based our comparison on the conserved second finger motif in MIG1, counting the number of identities to this finger for each finger in the other proteins. By this measure, the fingers of the two Egr proteins are most similar to MIG1, with 60% identical residues. The Wilms' tumour protein and Sp1 have 54% and 51% identities, respectively. The first two fingers of TFIIIA, a protein which is more distantly related to MIG1, have 38% identities. We conclude that MIG1, Sp1, and the Egr and Wilms' tumour proteins form a group of proteins with similar finger motifs. Within this group, MIG1 is most similar to the two Egr proteins.

Outside the fingers, MIG1 has no obvious similarity to other proteins. This absence of sequence conservation is a common finding among the C_2H_2 finger proteins; even the two Egr proteins, which have almost identical fingers, show little similarity to each other elsewhere. However, the non-finger region of MIG1 has several stretches of polyglutamine and glutamine alternating with asparagine (Figure 2). This motif has been found in a number of eukaryotic proteins that regulate gene expression (Schultz and Carlson, 1987). The non-finger region of MIG1 also contains a possible target site for cAMP dependent protein kinase (Krebs and Beavo, 1979), at amino acids 307–311.

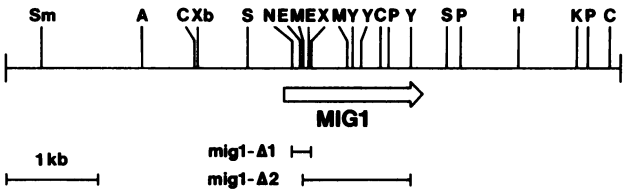

Fig. 1. Restriction map of the pMIG1 insert. The large arrow is the *MIG1* open reading frame. The *mig1-δ1* and *mig1-δ2* deletions, shown as bars below the map, were used in one-step gene disruptions (see Materials and methods). The *Eco*RI site of the pHR81 polylinker is located at the left end of the map. Abbreviations: A, *Apa*LI; C, *Cla*I; E, *Eco*RI; H, *Hin*dIII; K, *Kpn*I; M, *Mlu*I; N, *Nar*I; P, *Spe*I; S, *Sac*I; Sm, *Sma*I; X, *Xho*I; Xb, *Xba*I; Y, *Sty*I.

Overexpression of MIG1 inhibits carbohydrate catabolism

The *MIG1* gene was cloned by its ability to inhibit *GAL* gene expression in a strain carrying the *GAL1–TPK2* gene fusion. The inability of surviving cells to ferment galactose showed that the *GAL* genes were inhibited in these cells. To investigate the mechanism of inhibition, we proceeded to test the effect of pMIG1 on *GAL* gene expression in the isogenic wild-type strain W303-1A. Unexpectedly, W303-1A could ferment galactose in the presence of the plasmid, and Northern blots showed only a minor effect on the *GAL* genes (data not shown). Thus, *GAL* gene expression was not as strongly inhibited as in surviving cells from the *GAL1–TPK2* fusion strain. It is conceivable that plasmid copy number determines the degree of inhibition, with surviving cells having been selected for a copy number high enough to turn off the *GAL1* promoter. To test whether this was the case, we used the defective *LEU2-d* gene on the plasmid to select for a high copy number in W303-1A (Erhart and Hollenberg, 1983). We found that pMIG1 severely reduces glucose fermentation under these conditions. Growth on raffinose was also inhibited, while growth on glucose was unaffected (Figure 4A). Thus, both the *GAL* genes and *SUC2*, the active *SUC* gene in W303-1A, are repressed by pMIG1. Similar results were obtained when we tested the

Table I. Yeast strains

Strain	Genotype	Source
W303-1A	*MATa SUC2 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*	R.Rothstein
H174	*MATa SUC2 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 mig1-δ1::LEU2*	This work
H190	*MATa SUC2 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 mig1-δ2::LEU2*	This work
H250	*MATa SUC2 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 mig1-δ2::URA3*	This work
U670	*MATa SUC2 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 rad6::LEU2*	R.Rothstein
H211	*MATa SUC can1 gal2 his3-11,15 leu2-3,112 mig1-δ1::LEU2*	This work
H213	*MATα SUC ade5 cyh2 gal2 leu2-3,112 lys2-2 met13-c trp5-2 ura3-1*	This work
H243	*MATa SUC cyh2 gal2 leu2-3,112 met13-c trp5-2 ura3-1*	This work
H272	*MATα SUC can1-100 his3-11,15 leu2-3,112 ade mig1-δ2::URA3 rad6::LEU2 ura3-1*	This work
R277	*MATα SUC ade2-1 ade5 ade6 cly8 cyh2 his7-1 lys2-2 met13-c trp5-2 tyr1-2 ura3-1*	R.Rothstein
MCY835	*MATα SUC2 cid1-226 gal lys2-801 ura3-52*	M.Carlson

effect of pMIG1 on fermentation of maltose, melibiose and α-methylglucoside in strains carrying *MAL*, *MEL* and *MGL* genes (data not shown). We conclude that several genes involved in carbohydrate catabolism are inhibited by pMIG1 if the plasmid is present in a high copy number. These genes are subject to different forms of induction, but they are all repressed by glucose. This suggested that *MIG1* could be involved in glucose repression.

MIG1 is involved in glucose repression of the SUC2 gene

To further investigate the function of *MIG1*, we made disruptions of the gene (Rothstein, 1983). We found that *MIG1* is not required for growth in either diploids or haploids. Neither is it required for mating, sporulation or spore germination. Disruption of *MIG1* did not affect transcription of the *HIS3* or *URA3* genes (dat not shown). We proceeded to test the effect of *MIG1* gene disruptions on carbohydrate metabolism. The ability of the cell to grow on various carbon sources did not change. However, glucose repression of the *SUC2* gene was severely affected by the disruption. Thus, the *mig1*$^-$ strain H174 was able to grow on raffinose in the presence of 2-deoxyglucose (Figure 4B). 2-Deoxyglucose is a non-metabolized glucose analogue which inhibits *SUC2* expression in wild-type cells and therefore prevents their growth on raffinose (Zimmermann *et al.*, 1977). We conclude that glucose repression of *SUC2* is dependent on *MIG1*.

To quantify the effects of *MIG1* on glucose repression, we used Northern blots to measure *SUC2* mRNA in repressed cells (grown on glucose) and derepressed cells (grown on raffinose). We found that *SUC2* expression on glucose is nine times higher in the *mig1*$^-$ strain than in the wild-type, reaching half the wild-type level on raffinose (Table III). It should be noted that mRNA for the glucose-repressed secreted invertase is absent in wild-type cells grown on glucose. The *SUC2* mRNA in these cells is instead a minor constitutive species which encodes an intracellular form of the enzyme (Carlson and Botstein, 1982). The effect of *MIG1* on glucose repression of *SUC2* is therefore probably much more than 9-fold. We also found that disruption of

Table II. Tetrad data for the *mig1* locus

Interval	Ascus type			x_6 (cM)
	PD	NPD	T	
trp5–mig1	266	0	33	5.4
trp5–rad6	98	1	73	22.4
trp5–cyh2	85	10	209	47.1
mig1–rad6	111	1	62	19.4
mig1–cyh2	106	9	196	42.2
rad6–cyh2	87	0	87	25.1

The data include 142 tetrads from a cross of H211 to H213, and 176 tetrads from a cross of H243 to H272 (Table I). Map distances were corrected for high order events as described by Ma and Mortimer (1983).

```
AGAAGTTAGCGAGCCAGCACGTTTCCTTCGCGCCGGTTTTTTTTTTTATTTTTTATTTAATTTTTTTCAAAACTTGACGTGTAGAAGATTGACGTCTGGGGTTGGCCCCATGAATTTTTT  -721
ATCTATTTCTTCCCTGTGCCTAATTCGTAATATCTCCACTGATAGCGTAAAAAAGCGCTTTATTTTTCCGAAAGTTGTGATGGCATTCCATACAGTTTATAGGATGGTAGCAAGTGATAA  -601
GAGCTTGGGGCCCACAATTAAGCAGCAGAAAAGCGCAATTGCGACACTAGCAGTGTAACTCGATAGGATTTTATGGAGTGTTGATGAATGTAGTTGCCATTATAATGGCTTCTCAGGAAA  -481
CCTGTAGCGGCCGGGCCCCGCGAGAAACTCCAGAGTGGCGTAGGCCGGCTTGTTTAGTTGCTAGCATACTTGTTCGAGCTCTTGAGTTCTCCTGGCTTTTCTCCACGTGTCTGCTTGTTG  -361
CCTTTGATTTCGCGAGAGACTGCGGACTGCCACCCCACTCCGGGAAAACCTGGAGTGATGGTAAAGGCCCTGCGGCGCTTTCTTTCGGAAAAACTAATACGAAAGAAGCAACAACAAATT  -241
TTTACAACAGGCTGGACGAGAAAAGTGGGGAAGAGCAAGCAGAAAAAAGAAAAGAGAATAGTCGTTCTTGGATAATTTATTTATTATAACCCTTTTTTTTCACCCCAGTACTCATTAACG  -121
AAGACAAAGGAGAGTAAGAAAGCCCGGTAAAGCATTTCGAAGATAAGAGAGCCATTTATTCTAGCTCGCTTGTAACTACACGAGAGTTGAGTATAGTGGAGACGACATACTACCATAGCC  -1
 M  Q  S  P  Y  P  M  T  Q  V  S  N  V  D  D  G  S  L  L  K  E  S  K  S  K  S  K  V  A  A  K  S  E  A  P  R  P  H  A  C  40
ATGCAAAGCCCATATCCAATGACACAAGTGTCTAACGTTGATGATGGGTCACTATTGAAGGAGAGTAAAAGCAAGTCCAAAGTAGCTGCGAAGTCAGAGGCGCCAAGACCACATGCTTGT  120
 P  I  C  H  R  A  F  H  R  L  E  H  Q  T  R  H  M  R  I  H  T  G  E  K  P  H  A  C  D  F  P  G  C  V  K  R  F  S  R  S  80
CCTATCTGTCATAGAGCTTTTCACAGACTGGAACATCAGACGAGACACATGAGAATTCATACAGGTGAGAAGCCTCACGCGTGTGACTTCCCCGGATGTGTGAAAAGGTTCAGTAGAAGC  240
 D  E  L  T  R  H  R  R  I  H  T  N  S  H  P  R  G  K  R  G  R  K  K  K  V  V  G  S  P  I  N  S  A  S  S  S  A  T  S  I  120
GATGAACTGACGAGACACAGAAGAATTCATACAAACTCCCACCCTCGAGGTAAAAGAGGCAGAAAGAAGAAGGTTGTGGGCTCTCCAATAAATAGTGCTAGTTCTAGTGCTACCAGTATA  360
 P  D  L  N  T  A  N  F  S  P  P  L  P  Q  Q  H  L  S  P  L  I  P  I  A  I  A  P  K  E  N  S  S  R  S  S  T  R  K  G  R  160
CCAGATTTAAATACGGCAAATTTTTCACCGCCATTACCACAGCAACACCTATCGCCTTTAATTCCTATTGCTATTGCTCCGAAAGAAAATTCAAGTCGATCTTCTACAAGAAAAGGTAGA  480
 K  T  K  F  E  I  G  E  S  G  G  N  D  P  Y  M  V  S  S  P  K  T  M  A  K  I  P  V  S  V  K  P  P  P  S  L  A  L  N  N  200
AAAACCAAATTCGAAATCGGCGAAAGTGGTGGGAATGACCCATATATGGTTTCTTCTCCCAAAACGATGGCTAAGATTCCCGTCTCGGTGAAGCCTCCACCTTCTTTAGCACTGAATAAT  600
 M  N  Y  Q  T  S  S  A  S  T  A  L  S  S  L  S  N  S  H  S  G  S  R  L  K  L  N  A  L  S  S  L  Q  M  M  T  P  I  A  S  240
ATGAACTACCAAACTTCATCCGCTTCCACTGCTTTGTCTTCGTTGAGCAATAGCCATAGTGGCAGTAGACTGAAACTGAACGCGTTATCGTCCCTACAAATGATGACGCCCATTGCTAGC  720
 S  A  P  R  T  V  F  I  D  G  P  E  Q  K  Q  L  Q  Q  Q  Q  N  S  L  S  P  R  Y  S  N  T  V  I  L  P  R  P  R  S  L  T  280
AGTGCGCCAAGGACTGTTTTCATAGACGGTCCTGAACAGAAACAACTACAACAACAACAAAATTCTCTTTCACCACGTTATTCCAACACTGTTATATTACCAAGGCCGCGATCTTTAACG  840
 D  F  Q  G  L  N  N  A  N  P  N  N  N  G  S  L  R  A  Q  T  Q  S  S  V  Q  L  K  R  P  S  S  V  L  S  L  N  D  L  L  V  320
GATTTTCAAGGATTGAACAATGCAAATCCAAACAACAATGGAAGTCTCAGAGCACAAACTCAGAGTTCCGTACAGTTGAAGAGACCAAGTTCAGTTTTAAGTTTGAACGACTTGTTGGTT  960
 G  Q  R  N  T  N  E  S  D  S  D  F  T  T  G  G  E  D  E  E  D  G  L  K  D  P  S  N  S  S  I  D  N  L  E  Q  D  Y  L  Q  360
GGCCAAAGAAATACCAACGAATCTGACTCTGATTTTACTACTGGTGGTGAGGATGAAGAAGACGGACTAAAGGACCCGTCTAACTCTAGTATCGATAACCTTGAGCAAGACTATTTGCAA  1080
 E  Q  S  R  K  K  S  K  T  S  T  P  T  T  M  L  S  R  S  T  S  G  T  N  L  H  T  L  G  Y  V  M  N  Q  N  H  L  H  F  S  400
GAGCAATCAAGAAAGAAATCTAAGACTTCCACGCCCACGACAATGCTAAGTAGATCCACTAGTGGTACGAATTTGCACACTTTGGGGTATGTAATGAACCAAAATCACTTGCATTTCTCC  1200
 S  S  S  P  D  F  Q  K  E  L  N  N  R  L  L  N  V  Q  Q  Q  Q  Q  E  Q  H  T  L  L  Q  S  Q  N  T  S  N  Q  S  Q  N  Q  440
TCATCATCTCCTGATTTCCAAAAGGAGTTGAACAACAGATTACTGAACGTTCAACAACAGCAGCAAGAGCAACATACCCTACTGCAATCACAAAATACGTCAAACCAAAGTCAAAATCAA  1320
 N  Q  N  Q  M  M  A  S  S  S  S  L  S  T  T  P  L  L  L  S  P  R  V  N  M  I  N  T  A  I  S  T  Q  Q  T  P  I  S  Q  S  480
AATCAAAATCAAATGATGGCTTCCAGTAGTTCGTTAAGTACAACCCCGTTATTATTGTCACCAAGGGTGAATATGATTAATACTGCTATATCCACCCAACAAACCCCCATTTCTCAGTCG  1440
 D  S  Q  V  Q  E  L  E  T  L  P  P  I  R  S  L  P  L  P  F  P  H  M  D  504
GATTCACAAGTTCAAGAACTGGAAACATTACCACCCATAAGAAGTTTACCGTTGCCCTTCCCACACATGGACTGATACGCTGACAAGTTTTTGGCGGTGCAGATAAATCAAAAGACAATA  1560
GACAAGAATTAATAATATTAACAATTAATAATTAATAAATAATAAATAATAATAATAATAATAATAATAATAATAGTAATAATAATAATAATTAATAACGATAAAAATATTTAA  1680
```

Fig. 2. Sequence of the *MIG1* gene. The two finger motifs and three glutamine-rich stretches in the predicted MIG1 protein sequence are underlined. Also underlined is a TATTTA motif in the *MIG1* upstream region.

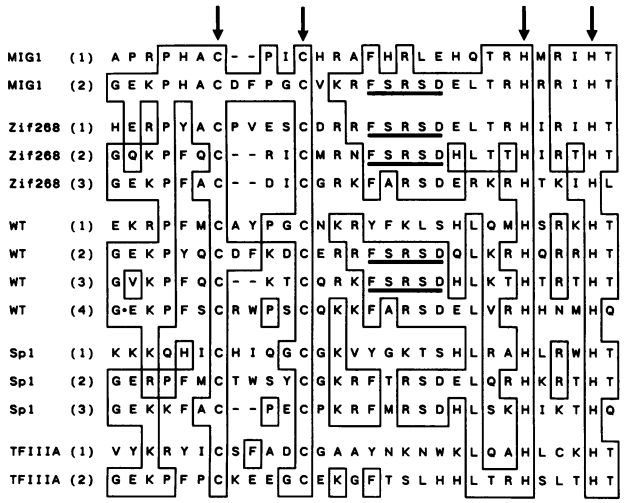

Fig. 3. Zinc fingers in the MIG1 protein. Alignment of MIG1 zinc fingers to other C_2H_2 finger proteins. Identities to the second finger of MIG1 are enclosed within boxes. The two Egr proteins have almost identical fingers; only those of Zif268 (Krox-24/Egr-1) are therefore shown. Also shown are the four fingers of the Wilms' tumour protein (WT), the three fingers of Sp1, and the first two fingers of TFIIIA. The arrows point to the cysteine and histidine residues that are conserved in all C_2H_2 zinc finger motifs. The fingertip motif FSRSD in the MIG1, Egr and Wilms' tumour proteins is underlined. The dot marks a polymorphism in the Wilms' tumour protein, where the sequence of Gessler *et al.* (1990) has an insertion of three residues not found in the sequence of Call *et al.* (1990). Numbers in parentheses refer to the number of the finger motif, counted from the amino terminus of the protein.

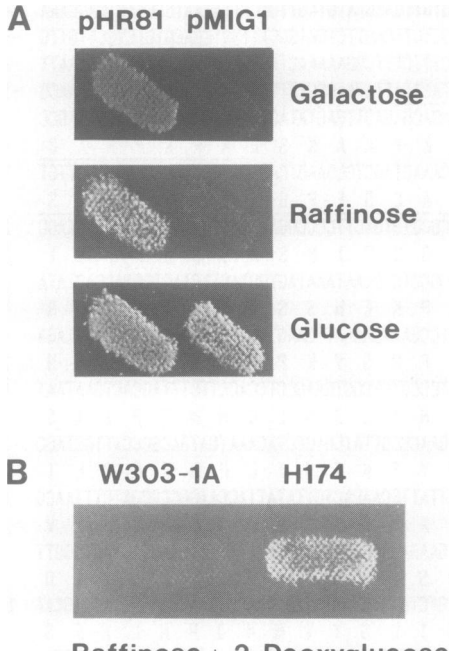

Fig. 4. Growth phenotypes of the *MIG1* gene. (**A**) Overexpression of *MIG1* inhibits the *GAL* and *SUC2* genes. W303-1A cells containing either pMIG1 or its parental vector pHR81 were grown on uracil-less glucose plates, and then replicated to leucine-less plates containing different carbon sources. Selection for leucine prototrophy was used to maintain a high plasmid copy number. (**B**) Disruption of *MIG1* makes the *SUC2* gene resistant to glucose repression. The isogenic strains W303-1A (*MIG1* $^+$) and H174 (*mig1* $^-$) were grown on glucose and then replicated to raffinose plates containing 200 μg/ml of 2-deoxyglucose.

Table III. Effect of *MIG1* on *SUC2* mRNA

Strain	Genotype	Carbon source	
		Glucose	Raffinose
W303-1A	*MIG1* $^+$	5.5	100.0
H174	*mig1* $^-$	49.1	208.8

SUC2 mRNA levels are shown as percent of the derepressed wild-type level (W303-1A grown on raffinose).

MIG1 doubles the *SUC2* mRNA level in cells grown on raffinose (Table III). This is probaby due to incomplete derepression in the wild-type cell, since raffinose degradation generates fructose, a repressing sugar. Elimination of glucose repression should therefore cause a further increase in *SUC2* expression on raffinose. Finally, there was a residual 4-fold difference in the *mig1* $^-$ strain between *SUC2* mRNA levels on glucose and raffinose (Table III). This suggests that mechanism(s) which are independent of *MIG1* may contribute to glucose repression of *SUC2*.

MIG1 protein binds to the SUC2 upstream region

The zinc fingers in MIG1 suggested that it could be a DNA binding protein. We therefore tested whether MIG1 could bind to the *SUC2* gene. The protein was made *in vitro* and its DNA binding ability was assayed by agarose gel shift. We found that MIG1 binds to the *SUC2* upstream region, between nucleotides −649 and −382 (Figure 5). This region is required for *SUC2* expression and can confer glucose repression to heterologous genes (Sarokin and Carlson, 1986). The gel shift was specific for MIG1; it was not seen with GAL4 protein or with mock-translated reticulocyte lysate (Figure 5). We proceeded to map the *MIG1* binding in *SUC2* by DNase I footprinting. We found two protected sites, one at positions −505 to −483 and one at −451 to −426 (sites A and B, Figure 6). Both sites contain similar GC-rich motifs, which are inverted with respect to each other. The patterns of protection are similar in that both sites are more extended on the C-rich strand of the motif. Both sites also have a single hypersensitive base on the G-rich strand, an adenine at the 3′ end of the motif. This is within the region which is protected on the C-rich strand. In addition, there are several hypersensitive regions flanking the two protected sites. This suggests that the DNA changes conformation when MIG1 is bound. A possible explanation could be formation of a loop due to cooperative binding at the two sites, as was shown to occur with lambda repressor (Griffith *et al.*, 1986).

For one of the Egr proteins, Zif268, several binding sites have been identified by DNase I footprinting (Christy and Nathans, 1989). Identical high affinity Zif268 sites, GCGGGGGCG, were found in the promoters of the *zif268*, *jun-D* and *475* genes. These genes also contain several low affinity sites, which differ in one or two positions from the high affinity nonamer. The second Egr protein, Krox-20, also binds to this motif (Chavrier *et al.*, 1990). The two MIG1 sites are very similar to the nonamer. The GC motif at MIG1 site B, CCGGGGGCG (Figure 6), differs from it in only one position. Site B is thus more conserved than the low affinity Zif268 sites in the *475* and *zif268* genes, which differ in two positions (Christy and Nathans, 1989). It is therefore possible that the Egr proteins and MIG1 recognize the same sequence, of which site B is an acceptable variant.

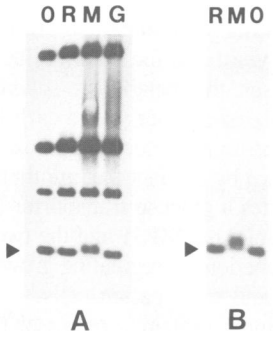

Fig. 5. Agarose gel shift assay. End-labelled *SUC2* fragments were incubated with MIG1 protein made *in vitro*, and then size fractionated on 2% agarose gels. (**A**) *Hin*dIII–*Eco*RI–*Sal*I fragments of pWJ210. The arrow points to a 426 bp *Sal*I–*Hin*dIII fragment containing *SUC2* DNA from −844 to −419, which shifts in the presence of MIG1 protein. (**B**) Gel shift of a purified 268 bp fragment containing *SUC2* DNA from −649 to −382. Abbreviations: G, GAL4 protein; M, MIG1 protein; R, mock-translated reticulocyte lysate; 0, no added protein.

This is supported by the fact that the G to C substitution in site B is also found in the low affinity Zif268 site of gene *475*. The GC motif at MIG1 site A, GCGGGG, is identical to the first two-thirds of the nonamer. It is conceivable that this motif is sufficient for binding, when flanked by acceptable nucleotides. This would be similar to Sp1, which binds with high affinity to a GGGGCGGGGC motif, but also with lower affinity to the core site GGGCGG (Kadonaga *et al.*, 1986).

Discussion

We have cloned a new yeast gene, *MIG1*, which is involved in glucose repression of the *SUC2* gene. *MIG1* does not seem to be allelic to any previously known gene involved in this process. Glucose repression of *SUC2* is affected by mutations in *HEX1*, *HEX2*, *SSN6*, *TUP1* and *CID1* (Neigeborn and Carlson, 1987). *HEX1*, *HEX2* and *SSN6* have all been cloned, and clearly differ from *MIG1* (Fröhlich *et al.*, 1984; Niederacher and Entian, 1987; Schultz and Carlson, 1987). The map position shows that *MIG1* differs from *TUP1* (Mortimer *et al.*, 1989). *CID1* has not been cloned or mapped, but pMIG1 failed to complement the *cid1-226* mutation in MCY835 (Table I), and a cross of this strain to a *mig1* strain gave 4:0, 3:1 and 2:2 segregation for 2-deoxyglucose-resistant growth on raffinose (data not shown). We therefore conclude that *MIG1* also differs from *CID1*.

We have shown that overexpression of *MIG1* inhibits *SUC2* expression, while disruption of *MIG1* interferes with glucose repression of *SUC2*. We have also shown that MIG1 protein binds to the *SUC2* upstream region. These findings suggest that MIG1 is a repressor of *SUC2* transcription. There is no previous case in which a C_2H_2 zinc finger protein has been implicated in repression rather than in activation of a target gene. However, two other families of transcription factors, the homeobox proteins and the C_x zinc finger proteins, contain both repressors and activators (Levine and Manley, 1989). Some of these proteins can function as either, depending on the circumstances. This raises the possibility that MIG1 could also function as an activator. The glutamine-rich motifs in MIG1 suggest that

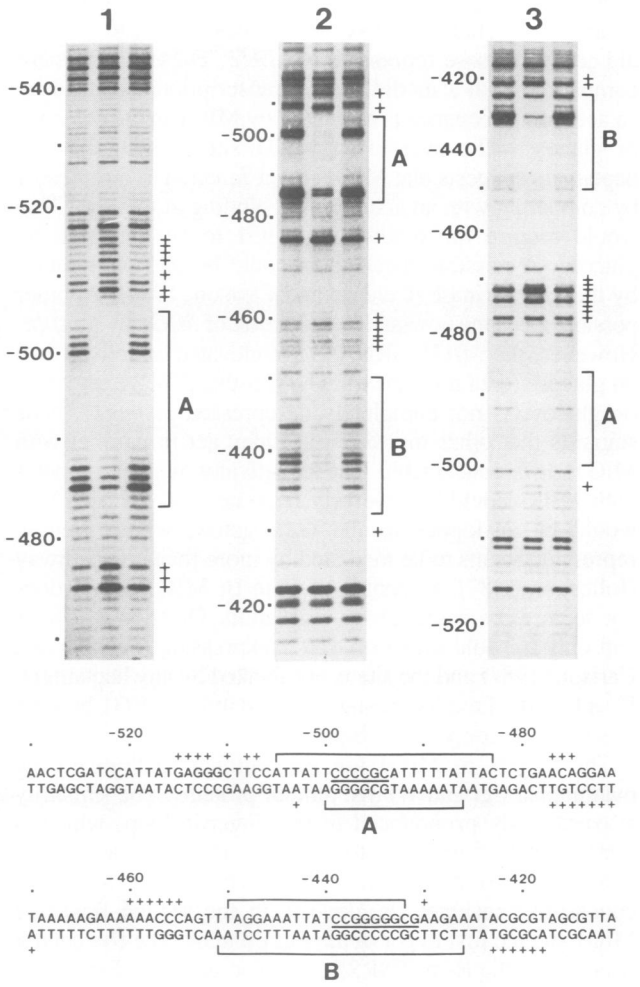

Fig. 6. Footprints of MIG1 on the *SUC2* upstream region. End-labelled DNA fragments from the *SUC2* upstream region were incubated with MIG1 protein (centre lanes) or mock-translated reticulocyte lysate (flanking lanes). The samples were then digested with DNase I and size fractionated on 6% denaturing polyacrylamide gels. (**1**) Coding strand, from the *Mlu*I site at −423. (**2**) Coding strand, from the *Nco*I site at −386. (**3**) Non-coding strand, from the *Cfr*13I site at −569. Protected and hypersensitive bases, as determined by densitometric scanning, are shown as brackets and plus signs, respectively. The GC motifs discussed in the text are underlined. The numbers are nucleotide positions relative to the start codon of secreted invertase (Sarokin and Carlson, 1986).

this may be the case, since a high content of glutamine is found in the activating domains of Sp1 (Courey and Tjian, 1988). It should be noted that the glucose response involves induction, as well as repression, of metabolic pathways. It is therefore conceivable that MIG1 could have an activating role for genes that are induced by glucose.

Upstream sequences that regulate *SUC2* expression have been studied by Sarokin and Carlson (1984, 1986). A heptamer motif, (A/C)(A/G)GAAAT, was suggested to be involved in activating transcription. The two MIG1 motifs differ from this sequence, but site B partially overlaps with two such heptamers. These are the only two heptamers in *SUC2* that match the above consensus sequence, and promoter fusion studies suggest that they may function as activating sequences. Thus, the 32 bp between −437 and −406, which include one of the heptamers but only part of MIG1 site B, could activate the *LEU2* promoter (Sarokin and Carlson, 1986). However, transcription was not

significantly repressed by glucose. In contrast, a fragment which also includes the two MIG1 sites (−542 to −384), did confer glucose repression to *LEU2*. These findings are compatible with a model where transcription mediated by an activating sequence is repressed by MIG1 in the presence of glucose. The overlap of MIG1 site B with the two heptamers suggests that MIG1 might function as a repressor by competing with an activator for binding at this site. This would require the binding of MIG1 to be regulated by glucose. A possible mechanism could be phosphorylation by the SNF1 kinase (Celenza and Carlson, 1986). Another possible mechanism would be induction of *MIG1* by glucose. However, the *MIG1* mRNA is not elevated in cells grown on glucose (data not shown). The fact that *SUC2* expression on glucose is not completely derepressed in *mig1*$^-$ cells suggests that other mechanism(s) may act in parallel with MIG1. It is conceivable that an activator which competes with MIG1 could be inversely regulated by glucose. This would be analogous to the *GAL* genes, where glucose repression seems to be mediated by more than one pathway (Johnston, 1987). In contrast to site B, MIG1 site A does not seem to be essential for regulation. Deletion of site A had only a 3-fold effect on *SUC2* expression (Sarokin and Carlson, 1984) and the site is not flanked by any heptamers. Possibly, the function of site A is to stabilize MIG1 binding at site B by cooperative binding.

The fingers of MIG1 are very similar to those in the mammalian Egr and Wilms' tumour proteins. The similarity is particularly pronounced in the fingertip loop, which is thought to be important for DNA binding (Miller *et al.*, 1985; Bellefroid *et al.*, 1989). The entire loop, 11 consecutive residues, is identical in the second finger of MIG1 and the first finger of the Egr proteins. The five central residues of the loop, FSRSD, are also conserved in finger 3 of the Egr proteins and in fingers 2 and 3 of the Wilms' tumour protein (Figure 3). These residues are thought to be at the tip of the finger (Berg, 1988; Parraga *et al.*, 1988), and are highly variable between different proteins. Thus, among 402 C_2H_2 zinc fingers now sequenced (Evans and Hollenberg, 1988; Bellefroid *et al.*, 1989; and references therein), the FSRSD motif occurs in only one more case: the developmental regulator br1A of *Aspergillus nidulans* (Adams *et al.*, 1988). Interestingly, Krox-20 and the Wilms' tumour protein have been proposed to be involved in developmental regulation (Wilkinson *et al.*, 1989; Gessler *et al.*, 1990). It should be noted that MIG1 and the Egr proteins, which share the FSRSD motif, recognize similar sites. In contrast, Sp1, which lacks the motif, binds to a different sequence. The specificity of the Wilms' tumour protein is not known, but the presence of the FSRSD motif suggests that it could be similar to that of MIG1 and the Egr proteins.

These similarities suggest that MIG1 and the mammalian proteins could also have similar functions. In particular, it is possible that the mammalian proteins, like MIG1, could function as repressors. It has already been suggested that the Egr proteins could be involved in down-regulating the early growth response genes, which are repressed soon after induction (Christy and Nathans, 1989). However, this would not rule out an activator role for the Egr proteins in other cases. In fact, it was shown recently that Krox-20 can function as an activator in *Drosophila* cells (Chavrier *et al.*, 1990). As discussed above, it is conceivable that MIG1 could have both repressing and activating functions in the glucose response. An interesting parallel can be drawn between the glucose response in yeast and the mammalian early growth response. In both cases, the metabolism is adapted for rapid growth, using glucose as an energy and carbon source. For mammalian cells, the importance of glucose utilization in this response is shown by the fact that another early growth response gene encodes a glucose transporter (Hiraki *et al.*, 1988). It is conceivable that MIG1 and the two Egr proteins could have similar functions in regulating growth and glucose utilization in yeast and mammalian cells.

For the Wilms' tumour protein, a repressor function would be consistent with its proposed role in development and tumorigenesis. Thus, Comings (1973) has suggested that tumour suppressor genes might encode repressors of transforming genes expressed during embryogenesis. It has been proposed that the Wilms' tumour gene could encode such a repressor, possibly acting on the *IGF2* gene (Knudson, 1986; Olshan, 1986). *IGF2* is expressed in Wilms' tumours at 10−100 times the adult level, using the fetal promoter (Reeve *et al.*, 1985; Scott *et al.*, 1985). Interestingly, the high affinity Sp1 site in this promoter (de Pagter-Holthuizen *et al.*, 1987) overlaps with a nonamer identical to MIG1 site B. Similar overlapping sites for Sp1 and Krox-20 were recently found in the *Hox-1.4* promoter (Chavrier *et al.*, 1990). A search of Genbank reveals that several mammalian genes contain overlapping Sp1 and MIG1/Egr sites. This raises the possibility that some mammalian genes could be regulated by competitive binding of activators and repressors at overlapping sites, similar to what we propose for *SUC2* regulation in yeast.

Materials and methods

Yeast strains and plasmids

Yeast strains H174, H190, H250 and U670 (Table I) were generated from strain W303-1A (Thomas and Rothstein, 1989) by one-step gene disruptions (Rothstein, 1983). Two deletions were used for the *MIG1* gene disruptions. One, *mig1-δ1*, spans the *Nar*I and *Xho*I sites and deletes the finger motifs. The other, *mig1-δ2*, goes from the 5′ *Mlu*I site to the 3′ *Sty*I site and deletes most of the *MIG1* open reading frame (Figure 1). Both disruptions have identical phenotypes. The inserted marker was either the *LEU2 Hpa*I−*Sal*I fragment or the *URA3 Hin*dIII fragment. Strains H211, H213, H243 and H272, used to map the *mig1* locus, were constructed in several crosses from strains H174, H250, U670 and R277 (Table I). Plasmid pWJ210 is pUC18 with the *URA3* gene in the *Hin*dIII site and a 1.45 kb *Xho*I−*Bam*HI fragment of pLS27δ-1900/-845,δ-418/-223 (Sarokin and Carlson, 1984) between the *Sal*I and *Bam*HI sites. Plasmid pHR81 and the yeast genomic library made in this vector have been described (Nehlin *et al.*, 1989).

Northern blots

Yeast cells were harvested in mid log phase, and RNA was isolated and separated on 0.8% agarose gels as described by Sherman *et al.* (1986). After transfer to Hybond-C extra membranes (Amersham, UK) the RNA was hybridized overnight at 42°C to ^{32}P-labelled probes in 50% formamide, 5 × Denhardt's solution, 5 × SSPE, 0.1% SDS and 100 μg/ml of salmon sperm DNA. The filters were washed for 20 min at 55°C in 0.2 × SSPE, 0.2% SDS. The *SUC2* probe was a 1.45 kb *Xho*I−*Bam*HI fragment of pLS27δ-1900/-845,δ-418/-223 (Sarokin and Carlson, 1984). Hybridizing mRNA was quantified by densitometric scanning of autoradiograms.

In vitro transcription and translation

A *Sca*I−*Sac*I fragment of pMIG1 containing the *MIG1* open reading frame was cloned into the *Bgl*II site of pSP64T (Krieg and Melton, 1984) to generate pJN35. RNA synthesis and capping was carried out with 5 μg of *Sal*I-linearized pJN35 in a total volume of 50 μl, using a Promega (Madison, WI) kit as described by the manufacturer (capping protocol 2). The RNA was precipitated with ammonium acetate/ethanol after the reaction, and then

translated in a rabbit reticulocyte lysate (Amersham, UK), using 2 μg of RNA with 40 μl of lysate and 50 μCi of [^{35}S]methionine. A single major 56 kd ^{35}S-labelled polypeptide was obtained, as predicted from the *MIG1* sequence (data not shown). PMSF was added to 4 mM, and the samples were stored at −70°C.

Agarose gel shift

In vitro translated MIG1 protein was incubated with ^{32}P-labelled restriction fragments for 30 min at 20°C in GN buffer without detergent (Xing and Worcel, 1989). The binding reactions contained 1 μl of reticulocyte lysate, ~100 pg of end-labelled DNA and 1 μg of poly(dI.dC) carrier DNA (Pharmacia LKB, Uppsala, Sweden), in a total volume of 10 μl. The samples were separated on 2% agarose gels as described by Berman *et al.* (1987), with 40 mM Tris/20 mM HAc, pH 8.0, 1 mM EGTA as electrophoresis buffer. The gels were run at 5 V/cm for 5 h at 20°C, dried, and subjected to autoradiography.

DNase I footprinting

The DNA used for footprinting was a 0.63 kb *Xho*I−*Hin*dIII fragment of pLS27δ-1900/-650 (Sarokin and Carlson, 1984) subcloned in pUC19. The fragment contains *SUC2* DNA from −649 to −22. For the footprint starting at the *Mlu*I site, the DNA was end-labelled at this site with [^{32}P]dCTP, using the Klenow enzyme. The plasmid was then recleaved within the pUC19 polylinker, and a 230 bp fragment was isolated by low melting point agarose gel electrophoresis. For the *Nco*I and *Cfr*13I sites, fragments starting at these sites were first subcloned in the *Sal*I site of pUC18. The adjacent *Hin*dIII site in the polylinker was then labelled, and recleaved fragments were isolated as above. DNA binding reactions were performed as in the gel shift assay, with 2 μl of lysate, 800 ng of carrier DNA and ~1 ng of ^{32}P-labelled DNA in a total volume of 20 μl. The samples were digested for 30 s with 400 ng of RNase A (to eliminate [^{35}S]methionyl-tRNA derived from the lysate) and then for 1 min at 26°C with 600 ng of DNase I (PL Biochemicals, Milwaukee, WI). The digestion was stopped by the addition of 60 μl of 40 mM Tris−HCl containing 20 mM EDTA, 0.5% SDS and 80 μg/ml tRNA. The samples were extracted with phenol, ethanol precipitated, and analysed by acrylamide gel electrophoresis. Protected and hypersensitive bases were identified by densitometric scanning of autoradiograms. This allowed a reproducible identification of some hypersensitive sites not easily detected by visual inspection. Nucleotide positions were identified by Maxam and Gilbert reactions run in parallel with the footprints.

Other methods

Yeast media were prepared according to Sherman *et al.* (1986), but with twice as much leucine. Galactose and raffinose media contained 2% glucose-free galactose or 3% raffinose (Sigma, St Louis, MO). To suppress non-specific aerobic growth, fermentation was scored in the presence of 20 μg/ml ethidium bromide (Johnston and Davis, 1984). Standard methods were used for cloning in *Escherichia coli* and yeast (Maniatis *et al.*, 1982; Rothstein, 1985), and for DNA sequencing (Sanger *et al.*, 1977; Maxam and Gilbert, 1980).

Acknowledgements

We thank Monika Carlberg and Anna Karin Tibell for excellent technical assistance, and Marian Carlson, Brehon Laurent, Rodney Rothstein and Lena Welsh for helpful comments and generous gifts of strains and plasmids. A gene identical to *MIG1* has been cloned by Klaus Huse, Stefan Hohmann, Eulogio Valentin and Friedrich Zimmermann. We thank them for making their sequence available for comparison prior to its publication. Finally, we are grateful for the advice provided by Niklas Dahl, Bo Ek, Jan Henriksson, Ann-Kristin Jonsson, Lars-Göran Josefsson and Dan Larhammar on various technical procedures.

References

Adams,T.H., Boylan,M.T. and Timberlake,W.E. (1988) *Cell*, **54**, 353−362.

Bellefroid,E.J., Lecocq,P.J., Benhida,A., Poncelet,D.A., Belayew,A. and Martial,J.A. (1989) *DNA*, **8**, 377−378.

Berg,J.M. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 99−102.

Berman,J., Eisenberg,S. and Tye,B.-K. (1987) *Methods Enzymol.*, **155**, 528−537.

Call,K.M., Glaser,T., Ito,C.Y., Buckler,A.J., Pelletier,J., Haber,D.A., Rose,E.A., Kral,A., Yeger,H., Lewis,W.H., Jones,C. and Housman,D.E. (1990) *Cell*, **60**, 509−520.

Carlson,M. (1987) *J. Bacteriol.*, **169**, 4873−4877.

Carlson,M. and Botstein,D. (1982) *Cell*, **28**, 145−154.

Celenza,J.L. and Carlson,M. (1986) *Science*, **233**, 1175−1180.

Chavrier,P., Zerial,M., Lemaire,P., Almendral,J., Bravo,R. and Charnay,P. (1988) *EMBO J.*, **7**, 29−35.

Chavrier,P., Vesque,C., Galliot,B., Vigneron,M., Dolle,P., Duboule,D. and Charnay,P. (1990) *EMBO J.*, **9**, 1209−1218.

Christy,B. and Nathans,D. (1989) *Proc. Natl. Acad. Sci. USA*, **86**, 8737−8741.

Christy,B.A., Lau,L.F. and Nathans,D. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 7857−7861.

Chu,G., Vollrath,D. and Davis,R.W. (1986) *Science*, **234**, 1582−1585.

Comings,D.E. (1973) *Proc. Natl. Acad. Sci. USA*, **70**, 3324−3328.

Courey,A.J. and Tjian,R. (1988) *Cell*, **55**, 887−898.

de Pagter-Holthuizen,P., Jansen,M., van Schaik,F.M.A., van der Kammen,R., Oosterwijk,C., Van der Brande,J.L. and Sussenbach,J.S. (1987) *FEBS Lett.*, **214**, 259−264.

Entian,K.-D. (1986) *Microbiol. Sci.*, **3**, 366−371.

Erhart,E. and Hollenberg,C.P. (1983) *J. Bacteriol.*, **156**, 625−635.

Evans,R.M. and Hollenberg,S.M. (1988) *Cell*, **52**, 1−3.

Fröhlich,K.-U., Entian,K.-D. and Mecke,D. (1984) *Mol. Gen. Genet.*, **194**, 144−148.

Gancedo,J.M. and Gancedo,C. (1986) *FEMS Microbiol. Rev.*, **32**, 179−187.

Gessler,M., Poustka,A., Cavenee,W., Neve,R.L., Orkin,S.H. and Bruns,G.A.P. (1990) *Nature*, **343**, 774−778.

Ginsberg,A.M., King,B.O. and Roeder,R.G. (1984) *Cell*, **39**, 479−489.

Griffith,J., Hochschild,A. and Ptashne,M. (1986) *Nature*, **322**, 750−752.

Harbury,P.A.B. and Struhl,K. (1989) *Mol. Cell. Biol.*, **9**, 5298−5304.

Hiraki,Y., Rosen,O. and Birnbaum,M.J. (1988) *J. Biol. Chem.*, **263**, 13655−13662.

Johnston,M. (1987) *Microbiol. Rev.*, **51**, 458−476.

Johnston,M. and Davis,R.W. (1984) *Mol. Cell. Biol.*, **4**, 1440−1448.

Joseph,L.J., Le Beau,M.M., Jamieson,G.A., Jr, Acharya,S., Shows,T.B., Rowley,J.D. and Sukhatme,V.P. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 7164−7168.

Kadonaga,J.T., Jones,K.A. and Tjian,R. (1986) *Trends Biochem. Sci.*, **11**, 20−23.

Kadonaga,J.T., Carner,K.R., Masiarz,F.R. and Tjian,R. (1987) *Cell*, **51**, 1079−1090.

Klug,A. and Rhodes,D. (1987) *Trends Biochem. Sci.*, **12**, 464−469.

Knudson,A.G., Jr (1986) *Annu. Rev. Genet.*, **20**, 231−251.

Krebs,E.G. and Beavo,J.A. (1979) *Annu. Rev. Biochem.*, **48**, 932−959.

Krieg,P.A. and Melton,D.A. (1984) *Nucleic Acids Res.*, **12**, 7057−7070.

Lemaire,P., Revelant,O., Bravo,R. and Charnay,P. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 4691−4695.

Levine,M. and Manley,J.L. (1989) *Cell*, **59**, 405−408.

Ma,C. and Mortimer,R.K. (1983) *Mol. Cell. Biol.*, **3**, 1886−1887.

Maniatis,T., Fritsch,E.F. and Sambrook,J. (1982) *Molecular Cloning. A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Maxam,A.M. and Gilbert,W. (1980) *Methods Enzymol.*, **65**, 499−560.

Milbrandt,J. (1987) *Science*, **238**, 797−799.

Miller,J., McLachlan,A.D. and Klug,A. (1985) *EMBO J.*, **4**, 1609−1614.

Mortimer,R.K., Schild,D., Contopolou,C.R. and Kans,J.A. (1989) *Yeast*, **5**, 321−404.

Nehlin,J.O., Carlberg,M. and Ronne,H. (1989) *Gene*, **85**, 313−319.

Neigeborn,L. and Carlson,M. (1987) *Genetics*, **115**, 247−253.

Niederacher,D. and Entian,K.-D. (1987) *Mol. Gen. Genet.*, **206**, 505−509.

Olshan,A.F. (1986) *Cancer Genet. Cytogenet.*, **21**, 303−307.

Parraga,G., Horvath,S.J., Eisen,A., Taylor,W.E., Hood,L., Young,E.T. and Klevit,R.E. (1988) *Science*, **241**, 1489−1492.

Reeve,A.E., Eccles,M.R., Wilkins,R.J., Bell,G.I. and Millow,L.J. (1985) *Nature*, **317**, 258−260.

Rothstein,R.J. (1983) *Methods Enzymol.*, **101**, 202−211.

Rothstein,R.J. (1985) In Glover,D.M. (ed.), *DNA Cloning—A Practical Approach*. IRL Press, Oxford, Vol. II, pp. 45−66.

Sanger,F., Nicklen,S. and Coulson,A.R. (1977) *Proc. Natl. Acad. Sci. USA*, **74**, 5463−5467.

Sarokin,L. and Carlson,M. (1984) *Mol. Cell. Biol.*, **4**, 2750−2757.

Sarokin,L. and Carlson,M. (1986) *Mol. Cell. Biol.*, **6**, 2324−2333.

Schultz,J. and Carlson,M. (1987) *Mol. Cell. Biol.*, **7**, 3637−3645.

Scott,J., Cowell,J., Robertson,M.E., Priestley,L.M., Wadey,R., Hopkins,B., Pritchard,J., Bell,G.I., Rall,L.B., Graham,C.F. and Knott,T.J. (1985) *Nature*, **317**, 260−262.

Sherman,F., Fink,G.R. and Hicks,J.B. (1986) *Methods in Yeast Genetics*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Sukhatme,V.P., Cao,X., Chang,L.C., Tsai-Morris,C.-H., Stamenkovich,

D., Ferreira,P.C.P., Cohen,D.R., Edwards,S.A., Shows,T.B., Curran,T., Le Beau,M.M. and Adamson,E.D. (1988) *Cell*, **53**, 37–43.
Thomas,B.J. and Rothstein,R. (1989) *Cell*, **56**, 619–630.
Toda,T., Cameron,S., Sass,P., Zoller,M. and Wigler,M. (1987) *Cell*, **50**, 277–287.
Wilkinson,D.G., Bhatt,S., Chavrier,P., Bravo,R. and Charnay,P. (1989) *Nature*, **337**, 461–464.
Xing,Y.Y. and Worcel,A. (1989) *Mol. Cell. Biol.*, **9**, 499–514.
Zimmermann,F.K. and Scheel,I. (1977) *Mol. Gen. Genet.*, **154**, 75–82.

Received on April 11, 1990; revised on May 30, 1990